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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

DEC - 2 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Salmonella typhimurium (Ames) Assay and Mouse Micro-nucleus Assay Mutagenicity Studies on Brodifacoum

FROM: Byron T. Backus, Ph.D., Toxicologist *Byron T. Backus*
Toxicology Branch 2
HED (7509C) 11/17/96

TO: John Redden,
HED (7509C)

THROUGH: K. Clark Swentzel *K. Clark Swentzel* 11/18/96
Section Head, Review Section II
Toxicology Branch 2
HED (7509C)

and

Viannakis Ioannou, Ph.D., Acting Branch Chief *Viannakis Ioannou*
Toxicology Branch 2
HED (7509C) 11/21/96

DP Barcode: D230214

Submission: n/a

Chemical: 112701 Brodifacoum

Action Requested: Review of a Salmonella typhimurium (Ames) Assay and Mouse Micronucleus Assay studies on brodifacoum.

EXECUTIVE SUMMARIES:

1. In independently performed microbial gene mutation assays (MRID No. 41563301), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 1.6-5000 µg/plate Brodifacoum (96.0%) in the absence or presence of S9 activation. Additional testing was carried out using a dose range of 0.064-5000 µg/plate -S9 and 0.064-200 µg/plate +S9 (strain TA1538) and 0.064-200 µg/plate +/- S9 (strain TA100). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.



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Compound insolubility was seen at 5000 $\mu\text{g}/\text{plate}$ +/-S9. Cytotoxicity was observed for the majority of strains at ≥ 40 $\mu\text{g}/\text{plate}$ -S9 and ≥ 1000 $\mu\text{g}/\text{plate}$ +S9. All strains responded in the expected manner to the nonactivated and S9-activated positive controls. There was, however, no convincing evidence that Brodifacoum induced a mutagenic response in any strain at any nonactivated or S9-activated dose.

The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

2. In a mouse micronucleus assay (MRID No. 415633902), groups of five male and five female C57BL/6J mice received single intraperitoneal injections of 0.187 or 0.30 mg/kg Brodifacoum (96%) prepared in corn oil. Doses evaluated represented 50 or 80% of the median lethal dose (MLD/7), respectively. Mice were sacrificed at 24, 48 and 72 hours postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

No deaths or other signs of toxicity were reported; however, 70% of the animals administered 0.5 mg/kg in a preliminary study died. There was also no evidence of target cell cytotoxicity. The positive control induced the expected high yield of MPEs in males and females. Brodifacoum did not induce a clastogenic or aneugenic effect in either sex at any dose or sacrifice time.

The study is classified as **Acceptable** and satisfies the requirements for FIFRA Test Guideline 84-2 for a micronucleus assay.

cc: Briscoe/Rubis

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BRODIFACOUM

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)
EPA Secondary Reviewer:
Byron T. Backus, Ph.D.
Review Section I,
Toxicology Branch II/HED (7509C)

SALMONELLA (84-2)

Signature: Nancy L. McCarroll
Date: 11/27/96

Signature: Byron T. Backus
Date: 11/27/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome
mutagenicity assay; OPPTS 870.5265 [§84-2]

DP BARCODE: D230214 **SUBMISSION NO.:** NONE

PC CODE: 112701 **TOX. CHEM. NO.:** **MRID NO:** 41563301

TEST MATERIAL (PURITY): Brodifacoum (96.0%)

SYNONYM(S): [3-(3-(4'-Bromo(1,1'-biphenyl)-4-yl)-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin]

CITATION: Callander, R.D (1983) Brodifacoum: An Evaluation in the Salmonella Mutagenicity Assay; Central Toxicology Laboratory: Alderley Park, Macclesfield, Cheshire, UK; Report No. CTL/P/949; Study Completion Date: October 21, 1983. (Unpublished) **MRID NUMBER:** 41563301

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In independently performed microbial gene mutation assays (MRID No. 41563301), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 1.6-5000 µg/plate Brodifacoum (96.0%) in the absence or presence of S9 activation. Additional testing was carried out using a dose range of 0.064-5000 µg/plate -S9 and 0.064-200 µg/plate + S9 (strain TA1538) and 0.064-200 µg/plate +/- S9 (strain TA100). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Compound insolubility was seen at 5000 µg/plate +/-S9. Cytotoxicity was observed for the majority of strains at ≥40 µg/plate -S9 and ≥1000 µg/plate +S9. All strains responded in the expected manner to the nonactivated and S9-activated positive controls. There was, however, no convincing evidence that Brodifacoum induced a mutagenic response in any strain at any nonactivated or S9-activated dose.

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Brodifacoum

Description: Not reported

Lot/batch number: Batch Reference No. 2051 (RS/78/A)

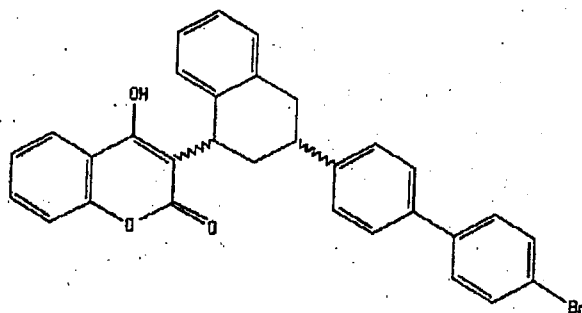
Purity: 96.0%

Receipt date: Not reported

Stability: Assumed to be stable under storage and test conditions.

CAS number: 56073-10-0

Structure:



Vehicle used: Dimethyl sulfoxide (DMSO)

Other provided information: Neither the test material storage conditions nor the frequency of dosing solution preparation were reported. Achieved concentrations were not verified.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO; the final concentration was not reported but was assumed to be 100 μ L/plate.

Positive:

Nonactivation:

N-Methyl-N'-nitro-n-nitroso-

guanidine (MNNG)

ICR 191

Daunorubicin (DR)

4-Nitro-o-phenylenediamine

(4NPD)

1, 2, 5 μ g/plate TA1535, TA1000.5, 1, 2 μ g/plate TA15370.5, 1, 2 μ g/plate TA981, 2, 5 μ g/plate TA1538Activation:

2-Aminoanthracene (2-AA)

0.5, 1, 2 μ g/plate TA1535, TA15370.2, 0.5, 1 μ g/plate TA1538, TA98,
TA100

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3. Activation: S9 derived from male Sprague Dawley (170-200 g) rats

<input checked="" type="checkbox"/> Aroclor 1254	<input checked="" type="checkbox"/> induced	<input checked="" type="checkbox"/> rat	<input checked="" type="checkbox"/> liver
<input type="checkbox"/> phenobarbital	<input type="checkbox"/> noninduced	<input type="checkbox"/> mouse	<input type="checkbox"/> lung
<input type="checkbox"/> none		<input type="checkbox"/> hamster	<input type="checkbox"/> other
<input type="checkbox"/> other		<input type="checkbox"/> other	

The rat liver S9 homogenate was prepared by the performing laboratory; separate batches were used for each experiment. The S9 mix contained the following components:

<u>Component:</u>	<u>Concentration</u>
Na ₂ HPO ₄	100 mM
KCl	33 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
MgCl ₂	8 mM
S9 homogenate	10 %

4. Test Organism Used: S. typhimurium strains

<input type="checkbox"/> TA97	<input checked="" type="checkbox"/> TA98	<input checked="" type="checkbox"/> TA100	<input type="checkbox"/> TA102
<input type="checkbox"/> TA104	<input checked="" type="checkbox"/> TA1535	<input checked="" type="checkbox"/> TA1537	<input checked="" type="checkbox"/> TA1538

list any others:

Test organisms were properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assay: Not performed.

b. Mutation assay: Three trials of the mutation assay were conducted using the following doses and strains:

Trials 1 and 2: 1.6, 8.0, 40, 200, 1000 or 5000 µg/plate +/-S9 -- all strains

Repeat trial: Owing to excessive cytotoxicity and a lack of a reproducible effect, the test was repeated with strain TA1538 and nonactivated concentrations of 0.064, 0.32, 1.6, 8.0, 40, 200, 1000 or 5000 µg/plate and S9-activated doses of 0.064, 0.32, 1.6, 8.0, 40 and 200 µg/plate. A repeat test was also performed with strain TA100 and doses of 0.064, 0.32, 1.6, 8.0, 40 and 200 µg/plate +/-S9.

In all trials, triplicate plates were prepared per dose per strain per condition. Duplicate plates were prepared for each positive control set and five replicates were prepared for each negative/solvent control set.

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B. TEST PERFORMANCE:1. Type of Salmonella Assay:

- ☒ Standard plate test
- ☐ Pre-incubation () minutes
- ☐ "Prival" modification
- ☐ Spot test
- ☐ Other (describe)

2. Mutation Assays: Briefly, 2-mL volumes of top agar were added to bijou bottles containing 0.1 mL of an overnight broth culture of the appropriate tester strain, 0.5 mL of sucrose-tris-EDTA S9 buffer (nonactivated tests) or 0.5 mL of S9 mix (S9-activated tests) and 0.1 mL of the appropriate test material dose, solvent, or positive control. The contents of the bottles were mixed, poured over Vogel-Bonner minimal medium, and incubated at 37°C for 64-68 hours. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were determined. Positive plates were verified by replica plating.

3. Evaluation Criteria:

- (a) Assay validity: The assay was considered valid if the positive controls induced a ≥ 2 -fold increase in revertant colonies of the corresponding tester strain and the response was dose related.
- (b) Positive response: The test material was considered positive if it caused a reproducible, statistically significant, ≥ 2 -fold and dose-related increase in mean revertant colonies/plate for at least one dose level.

4. Statistical Methods: The data were evaluated for statistical significance at $p < 0.05$ using a one-tailed Student's t-test.

C. REPORTED RESULTS: Doses ranging from 1.6-5000 $\mu\text{g}/\text{plate}$ +/-S9 were evaluated in Trials 1 and 2; results were as follows:

1. Trial 1: Representative findings from Trial 1 are presented in Table 1. As shown, compound precipitation was observed on plates containing the high dose (5000 $\mu\text{g}/\text{plate}$ +/-S9) and also at 1000 $\mu\text{g}/\text{plate}$ -S9. Cytotoxicity was apparent for strains TA1535, TA1537 and TA100 at nonactivated concentrations $\geq 40 \mu\text{g}/\text{plate}$ and for the majority of strains at $\geq 1000 \mu\text{g}/\text{plate}$ +S9. No clearly dose-related increases in mutant colonies of strains TA1535, TA1537, TA98 or TA100 were noted either in the absence or presence of S9 activation. Although an ~ 14.6 -fold increase in histidine revertants (his⁺) was calculated for strain TA1538 at 5000 $\mu\text{g}/\text{plate}$ -S9, the study author stated that replica plating of these "positive plates" verified that these colonies were not protrophic revertants. The data, therefore, indicate a cytotoxic rather than a mutagenic response. Sporadic increases in the colony counts of TA1538

were also seen at lower concentrations (4.9-fold at 200 $\mu\text{g}/\text{plate}$ and 3.7-fold at 8 $\mu\text{g}/\text{plate}$). However, revertant counts for intermediate levels (40 and 1000 $\mu\text{g}/\text{plate}$) were comparable or less than control. The lack of dose dependency and the low background spontaneous reversion rate for strain TA1538 (7.0 colonies without S9; 12.8 colonies with S9) were also noted by our reviewers. Despite the low spontaneous reversion frequency, the strain responded in the expected manner to the positive controls. Findings with TA1538 in the presence of S9 activation were not indicative of mutagenesis.

2. Trial 2: Data from Trial 2 show that nonactivated Brodifacoum was cytotoxic but not mutagenic in strains TA1535, TA1537, TA1538 and TA100 and neither cytotoxic nor mutagenic in strain TA98 (Table 2). The low background frequency for TA1538 was again noted. Results from the S9-activated phase of Trial 2 were in good agreement with the earlier findings indicating that doses ≥ 1000 $\mu\text{g}/\text{plate}$ were cytotoxic to the majority of strains and that the S9-activated test material was not mutagenic.
3. Repeat Tests: Owing to the increases in his⁺ colonies of strain TA1538 at intermediate levels in Trial 1 and the severe cytotoxicity noted for this strain in both trials, the nonactivated phase of testing was repeated with doses of 0.064-5000 $\mu\text{g}/\text{plate}$ -S9. S9-activated levels of 0.064-200 $\mu\text{g}/\text{plate}$ +S9 were also included in the repeat trial. Representative results from this repeat test are presented in Table 3. With the exception of the high dose, fold increases either approaching a doubling or equal to a 2-fold increase in his⁺ colonies of strain TA1538 were calculated over the entire nonactivated dose range. As previously noted, however, the spontaneous revertant frequency (7.8 colonies) was low. There was no evidence of a mutagenic response in strain TA1538 in the S9-activated phase of testing. Owing to severe cytotoxicity observed in Trials 1 and 2, strain TA100 was included in the repeat test; representative data are also presented in Table 3. As shown, findings with strain TA100 were negative.

In all trials, the tester strains responded to the mutagenic action of the corresponding nonactivated and S9-activated positive controls. Based on the overall results, the study author concluded that Brodifacoum was not mutagenic in this microbial test system.

- D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study author's conclusion that Brodifacoum was not mutagenic in this microbial gene mutation assay. The test material was investigated up to insoluble levels (5000 $\mu\text{g}/\text{plate}$ +/-S9), induced cytotoxicity for the majority of strains at levels ≥ 40 $\mu\text{g}/\text{plate}$ -S9 or ≥ 1000 $\mu\text{g}/\text{plate}$ +S9 but was not mutagenic. Although increases in the revertant colony counts either approaching a doubling or equal to a 2-fold increase over background did occur with strain TA1538 in the nonactivated phases of the study, there was no evidence of dose dependency and increases in the derivative strain of TA1538 (TA98) were not observed. Similarly, the ~ 14.6 -fold increase in the colony count calculated for strain TA1538 at 5000 $\mu\text{g}/\text{plate}$ -S9 in Trial 1 was clearly shown by replica plating to be a cytotoxic rather than a mutagenic response.

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Hence, we conclude that these increases were either anomalous or the consequence of severe cytotoxicity and, therefore, not indicative of mutagenicity. Although spontaneous revertant rates were consistently low for strain TA1538, results with the positive controls confirmed the sensitivity of the this strain to detect a mutagenic response. Additionally, the remaining tester strains responded in the expected manner to the appropriated nonactivated or S9-activated positive controls. Based on these considerations, we conclude that the study provided acceptable evidence that Brodifacoum was not mutagenic in this test system.

E. STUDY DEFICIENCIES: NONE.

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SALMONELLA (84-2)

TABLE 1. Representative Results of the Microbial/Mammalian Microsome Mutation Assay with Brodifacoum -- Trial 1

Revertants per Plate of Microbial Tester Strains ^a							
Substance	Dose per Plate	S9 Activation	<u>S. typhimurium</u>				
			TA1535	TA1537	TA1538	TA98	TA100
<u>Solvent Control</u>							
DMSO	100 µL	-					
	100 µL	+	13.6 17.4	6.2 7.2	7.0 12.8	19.2 25.6	94.8 107.0
<u>Positive Controls^b</u>							
MNNG	5 µg	-	2831.5	--	--	--	2223.5
ICR 191	1 µg	-	--	132.0	--	--	--
4NPD	5 µg	-	--	--	385.5	--	--
DR	2 µg	-	--	--	--	119.5	--
2AA	2 µg	+	122.5	123.0	--	--	--
	1 µg	+	78.5	36.5	549.5	476.5	686.5
<u>Test Material</u>							
<u>Brodifacoum</u>							
8 µg ^c	8 µg ^c	-	9.3	7.3	25.7(3.7) ^d	17.3	87.3
	40 µg	-	6.7	2.3	5.0	15.3	37.7
	1000 µg	-	4.0	5.7	9.0	14.7	21.7
	5000 µg ^e	-	3.7	5.3	102.3(14.6)	12.7	20.0
8 µg ^c	8 µg ^c	+	12.7	12.0	19.7	23.7	105.0
	40 µg	+	11.7	10.0	23.7	23.7	83.7
	1000 µg	+	6.0	5.0	10.7	15.7	29.0
	5000 µg ^e	+	5.3	1.7	9.7	14.3	37.0

^aMeans and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses. Three levels of each positive control were assayed; results for all strains were generally positive at the majority of doses. The presented data were selected as representative.

^bResults for the lowest assayed dose (1.6 µg/plate +/-S9) and an intermediate dose (200.0 µg/plate +/-S9) did not suggest a mutagenic effect. Values in () are fold increases over background.

^cCompound precipitation was reported at 1000 µg/plate -S9 and at 5000 µg/plate +/-S9.

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-nitrosoguanidine

4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin

2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, Tables 1 and 4; pp. 16, 17 and 21-23.

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TABLE 2. Representative Results of the Microbial/Mammalian Microsome Mutation Assay with Brodifacoum -- Trial 2

Revertants per Plate of Microbial Tester Strains ^a <i>S. typhimurium</i>							
Substance	Dose per Plate	S9 Activation	TA1535	TA1537	TA1538	TA98	TA100
<u>Solvent Control</u>							
DMSO	100 µL	-					
	100 µL	+	10.4	7.8	6.4	14.8	77.6
			12.6	10.6	11.8	20.0	89.8
<u>Positive Controls^b</u>							
MNNG	5 µg	-	3121.0	--	--	--	2815.5
ICR 191	1 µg	-	--	116.0	--	--	--
4NPD	5 µg	-	--	--	423.5	--	--
DR	2 µg	-	--	--	--	148.5	--
2AA	2 µg	+	145.0	161.0	--	--	--
	1 µg	+	63.5	51.0	545.5	505.0	620.0
<u>Test Material</u>							
<u>Brodifacoum</u>							
	8 µg ^c	-	8.0	5.7	5.0	16.5	64.7
	40 µg	-	10.7	1.3	3.7	17.3	29.7
	1000 µg	-	2.7	3.3	3.3	15.3	15.7
	5000 µg ^d	-	3.3	2.7	1.3	14.3	16.3
	8 µg ^c	+	9.3	7.7	14.5	23.0	86.3
	40 µg	+	8.3	7.7	10.3	17.0	65.0
	1000 µg	+	7.7	5.7	10.3	13.3	27.3
	5000 µg ^d	+	8.7	4.7	4.7	11.7	27.3

^aMeans and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses selected as representative.

^bResults for the lowest assayed dose (1.6 µg/plate +/-S9) and an intermediate dose (200.0 µg/plate +/-S9) did not suggest a mutagenic effect.

^cCompound precipitation was reported at 5000 µg/plate (Trial 2) and at 1000 and 5000 µg/plate (Repeat Trial).

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanidine

4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin

2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, Tables 2, 3, 5 and 6; pp. 18, 19 and 24-27.

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TABLE 3. Representative Results of the Microbial/Mammalian Microsome Mutation Assay with Brodifacoum -- Repeat Trial

Revertants per Plate of Microbial Tester Strains ^a <i>S. typhimurium</i>				
Substance	Dose per Plate	S9 Activation	TA1538	TA100
<u>Solvent Control</u>				
DMSO	100 μ L	-	7.8	89.4
	100 μ L	+	12.8	92.2
<u>Positive Controls^b</u>				
MNNG	5 μ g	-	--	1839.0
4NPD	5 μ g	-	380.0	--
2AA	1 μ g	+	157.0	230.0
<u>Test Material</u>				
Brodifacoum	1.6 μ g ^c	-	13.3(1.7) ^d	99.3
	8 μ g	-	14.0(1.8)	90.7
	40 μ g	-	15.3(2.0)	54.7
	200 μ g	-	14.7(1.9)	22.0
	1000 μ g	-	13.3(1.7)	ND
	5000 μ g ^e	-	10.0(1.3)	ND
	40 μ g ^c	+	15.0	106.7
	200 μ g ^e	+	11.7	73.7

^aMeans and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses

^bThree levels of each positive control were assayed; results for all strains were generally positive at the majority of doses. The presented data were selected as representative.

^cResults for lower doses (0.064 or 0.32 μ g/plate +/-S9 or 1.6 and 8.0 μ g/plate +S9) did not suggest a mutagenic effect.

^dValues in () are fold increases over background.

^eHighest assayed dose.

Abbreviations:

DMSO = Dimethyl sulfoxide

4NPD = 4-Nitro-O-phenylenediamine

2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, Tables 3 and 6; pp. 20 and 27.

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EPA Reviewer: Nancy McCarroll
 Review Section III,
 Toxicology Branch II/HED (7509C)
 EPA Secondary Reviewer:
Byron T. Backus, Ph.D.
 Review Section I,
 Toxicology Branch II/HED (7509C)

MICRONUCLEUS (84-2)

Signature: Nancy McCarroll
 Date: 11/7/96

Signature: Byron T. Backus
 Date: 11/17/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mouse micronucleus assay; OPPTS 870.5395 [84-2]

DP BARCODE: D230214 **SUBMISSION NO.:** NONE

PC CODE: 112701 **TOX. CHEM. NO.:** **MRID NO:** 41563302

TEST MATERIAL (PURITY): Brodifacoum (96%)

SYNONYM(S): [3-(3-(4'-Bromo(1,1'-biphenyl)-4-yl)-1,2,3,4-tetrahydro-1-naphthyl)-4-hydroxycoumarin]

CITATION: Sheldon, T., Richardson, C.R. and Shaw, J. (1983) Brodifacoum: An Evaluation of Brodifacoum in the Mouse Micronucleus Test; Central Toxicology Laboratory; Alderley Park, Macclesfield, Cheshire, UK; Report No. CTL/P/1006; Study Completion Date: November 25, 1983. (Unpublished) **MRID NUMBER:** 41563302

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No. 41563302), groups of five male and five female C57BL/6J mice received single intraperitoneal injections of 0.187 or 0.30 mg/kg Brodifacoum (96%) prepared in corn oil. Doses evaluated represented 50 or 80% of the median lethal dose (MLD/7), respectively. Mice were sacrificed at 24, 48 and 72 hours postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

No deaths or other signs of toxicity were reported; however, 70% of the animals administered 0.5 mg/kg in a preliminary study died. There was also no evidence of target cell cytotoxicity. The positive control induced the expected high yield of MPEs in males and females. Brodifacoum did not induce a clastogenic or aneugenic effect in either sex at any dose or sacrifice time.

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a micronucleus assay.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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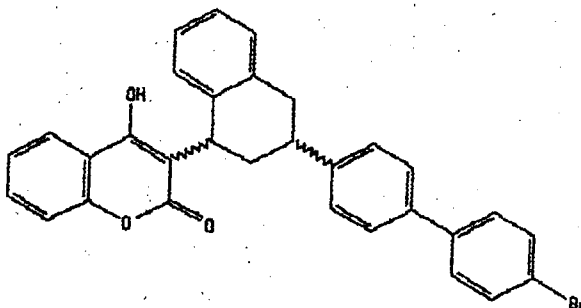
MICRONUCLEUS

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Brodifacoum

Description: Off-white powder
Lot/batch number: Batch Reference No. 2051 (RS/78/A)
Purity: 96%
Receipt date: Not reported
Stability: Not listed
CAS number: 56073-10-0
Structure:



Vehicle used: Corn oil

Other provided information: Neither the test material st conditions nor the frequency of dosing solution preparation included in the report. Achieved concentrations were not verif

2. Control Materials:

Negative/Route of administration: None

Vehicle/Final concentration/Route of administration: Corn oil administered once by intraperitoneal (ip) injection at a dosing v of 10 mL/kg.

Positive/Final concentration/Route of administration: Cyclophosphamide (CP) was prepared in corn oil and administered ip dose 65 mg/kg.

3. Test Compound:

Route of administration: IP

Dose levels used:

(a) Range-finding Test (Phase I): 0.05, 0.10, 0.15, 0.25 or

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MICRONUCLEUS (84-2)

- (b) Micronucleus assay: 0.187 or 0.30 mg/kg (5♂ and 5♀/group/
sacrifice)

4. Test Animals:

- (a) Species Mouse Strain C57BL/6J Age 8-12 weeks
Weight range: Not reported
Source: Animal Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK

(b) No. animals used per dose:

- (1) Range-finding test: 5 males; 5 females
(2) Micronucleus assay: 15 males; 15 females (all treatment groups)

(c) Properly maintained? YES

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

- (a) Test compound, vehicle and positive control:
Dosing: x once twice (24 hr apart)
N/A other (describe):
- (b) Sampling (after last dose): 6 hr 12 hr
x 24 hr x 48 hr x 72 hr

2. Tissues and Cells Examined:

x bone marrow N/A others (list):
Number of polychromatic erythrocytes (PCEs) examined per
animal: 500
Number of normochromatic erythrocytes (NCEs, more mature
RBCs) examined per animal: Not reported

3. Details of Slide Preparation: At 24, 48 or 72 hours after
administration of the test material, vehicle or positive control, the
appropriate groups of animals were sacrificed by cervical dislocation.
Bone marrow cells were collected from the femurs by dipping a fine paint
brush wetted with albumin into the bone marrow canal. Recovered cells
were spread onto slides and slides were stained with polychrome
methylene blue and eosin and scored. Slides were coded prior to
scoring.

4. Statistical Methods: The data were evaluated for statistical
significance using a one-sided Student's t-test at $p < 0.01$.

5. Evaluation Criteria: No criteria were provided to establish the
validity of the assay or the biological significance of the results.

BRODIFACOUM

MICRONUCLEUS (84-2)

C. REPORTED RESULTS:

1. Range-Finding Test: Groups of five male and five female mice received single ip injections of 0.05, 0.10, 0.15, 0.25 or 0.5 mg/kg and were observed daily for 7 days. Seven high-dose animals (5 males and 2 females) died. No deaths were noted in the lower treatment groups and no other signs of compound toxicity were reported. From these data, the median lethal dose (MLD/7) was estimated to be 0.375 mg/kg. Accordingly, doses selected for the micronucleus assay (0.187 and 0.30 mg/kg) represented 50 and 80% of the MLD/7, respectively.
2. Micronucleus Assay: No deaths or other clinical signs of toxicity were reported. Representative results presented in Table 1 show that the percentage of PCEs for males and females of the test material dose groups were generally comparable to the values for the vehicle control group at all harvest times. Similarly, no significant increase in the frequency of MPEs was observed at any harvest time in either males or females treated with Brodifacoum. By contrast to the uniformly negative results with the test material, the positive control (65 mg/kg CP) induced a clear increase in the frequency of MPEs in both sexes; the data combined for both sexes was significant ($p < 0.01$) at 24 and 48 hours.

Based on the findings, the study authors concluded that Brodifacoum was not genotoxic in this in vivo mouse micronucleus assay.

- D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that while the number of PCEs per animal that were scored for MPEs (500) was less than the recommended number (1000-2000 PCEs/animal), there was no convincing evidence that the test substance induced a clastogenic or aneugenic effect in either sex at any dose or sacrifice time. We conclude, therefore, that Brodifacoum was assayed to 80% of the MLD and failed to elicit a genotoxic response in the treated animals. The results obtained with the positive control (65 mg/kg CP) demonstrate that the sensitivity of test system to detect a positive effect was adequate. Hence, the study provided acceptable evidence that Brodifacoum was negative in this in vivo assay.

- E. STUDY DEFICIENCIES: See above regarding the number of PCEs analyzed per animal.

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with Brodifacoum

Substance	Dose per kg	Exposure Time* (hours)	Animals Analyzed per Group	MPEs/ 1000 PCEs*	% PCE
<u>Vehicle Control</u>					
Corn oil	10 mL	24	10	1.2	48.3
		48	10	3.2	43.6
		72	10	3.4	42.5
<u>Positive Control</u>					
Cyclophosphamide	65 mg	24	10	16.2*	41.3
		48	10	13.4*	38.4
		72	10	2.0	38.4
<u>Test Material</u>					
Brodifacoum	0.187 mg	24	10	1.8	51.0
		48	10	1.7	38.6
		72	10	1.8	49.1
	0.30 mg	24	10	2.2	40.0
		48	10	2.4	44.9
		72	10	1.1	36.6

*Time after administration of the test material, vehicle or positive control by intraperitoneal injection.
 *A total of 5000 PCEs were examined per group (500 PCEs/animal).

Abbreviations:

PCE = Polychromatic erythrocyte
 MCE = Micronucleated polychromatic erythrocyte
 NCE = Normochromatic erythrocyte

*Data combined for both sexes were significantly higher ($p < 0.01$) than the corresponding vehicle control group.
 Note: Data were extracted from the Study Report, Tables 1 and 2; pp 17 and 18.